Mantle and gill fine structure in the freshwater Asiatic clam, *Corbicula fluminea* (Müller)

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Keywords : *Corbicula fluminea*, Asiatic clam, mantle, gill, structure.

The epithelia of the main organs likely to be involved in contaminant uptake, viz. mantle and gill were studied in the Asiatic clam *Corbicula fluminea* as a base for future ecotoxicological studies. On its margin, the mantle epithelium displays three folds separated by two grooves. The outer epithelium made of cubic cells, and the periostracal groove are involved in the formation of the shell, secreting crystals and the periostracal lamella respectively. In the median area, the inner epithelium is composed of three cell types: ordinary epithelial cells, ciliated cells and mucocytes. The gill epithelium displays two structurally and functionally different areas: a respiratory area in the interlamellar chamber and, on the opposite side, a ciliated epithelium. The respiratory epithelium is composed of thin pavement epithelial cells. The ciliated epithelium is made of different cell types: lateral ciliated epithelial cells, secretory cells, latero-frontal ciliated cells and ciliated frontal cells. The role of the secretory cells is particularly discussed in relation to their structural similarity with lower vertebrate chloride cells (ionocytes) involved in iono- and osmoregulation processes.

Structure fine du manteau et de la branchie chez le bivalve asiatique, *Corbicula fluminea* (Müller)

Mots clés : *Corbicula fluminea*, bivalve, manteau, branchie, structure.

Les épithéliums des principaux organes susceptibles d'être impliqués dans la contamination par les contaminants aquatiques, le manteau et la branchie, ont été étudiés chez *Corbicula fluminea* dans le but d'utiliser ces connaissances dans de futures études écotoxicologiques. Dans sa partie marginale, le manteau présente trois replis séparés par deux sillons. L'épithélium le plus externe, composé de cellules cubiques, et le sillon périostacal sont impliqués dans la formation de la coquille par la sécrétion, respectivement, de cristaux et du périostacum. Dans la zone médiane, l'épithélium interne est formé de trois types cellulaires : cellules épithéliales ordinaires, cellules ciliées et mucocytes. L'épithélium branchial présente deux zones distinctes du point de vue structural et fonctionnel : une zone respiratoire dans la chambre interlamellaire, et à l'opposé, un épithélium cilié. L'épithélium respiratoire est formé de cellules épithéliales pavimenteuses. L'épithélium cilié présente différents types cellulaires : cellules épithéliales ciliées latérales, cellules sécrétrices, cellules ciliées latéro-frontales et cellules ciliées frontales. Le rôle des cellules sécrétrices est particulièrement discuté à cause de leur similarité de structure avec les cellules à chlorures (ou ionocytes) des vertébrés inférieurs impliquées dans les mécanismes d'iono- et d'osmorégulation.

1. Introduction

The freshwater Asiatic clam *Corbicula fluminea* is an invasive species originating from China (Britton & Morton 1982). Since the first published record of this

species in North America, it has become a major component of benthic communities in most lotic and lentic habitats, settling the sediment superficial layers. Most of the populations reach high densities, close to thousands per m². *Corbicula fluminea* is described as a pest with very severe economic and ecological effects, due to clog of water intakes, electric power plant cooling systems and sewage treatment plants (McMahon 1982, McCloskey et al. 1995). In the last decade, its presence was recorded in the South-West of France, downstream the Dordogne river (Mouthon 1981). This species has presently invaded the whole Garonne river

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hydrographic network as well as several lakes near the Aquitaine coast. It is likely to be contaminated by waterborne aquatic pollutants as well as those adsorbed and/or absorbed on inert and living particles (bacteria, phytoplankton...). It is now well established that bivalves, as filtering organisms, accumulate aquatic xenobiotics (Palmork & Solbakken 1981, Ishii et al. 1992, Viarengo & Nott 1993, Zarogian et al. 1993). Their ability to concentrate metals has been studied in marine (Skul'sky et al. 1989, Abbe & Sanders 1990, Roesijadi & Unger 1993, Abbe et al. 1994) and freshwater species (Doherty et al. 1988, Campbell & Evans 1991, Couillard et al. 1993, Mersch & Johansson 1993, Mersch et al. 1993, Tessier et al. 1993). However, so far, little is known about the actual effects of the accumulated metals on the organisms, and particularly, very few studies consider the structural and ultrastructural effects on the main organs in freshwater bivalves.

Moreover, from a more general point of view, few studies exist on the histological and cytological organisation of most organs and tissues in bivalves, particularly in freshwater species. Most recent work on gill structure deals with ciliature contributing to a better knowledge of feeding mechanisms in these species, but so far, the present knowledge of this biological barrier is mainly based upon studies performed in the late nineteenth (Peck 1877) and early twenties centuries (reviewed in Beninger et al. 1988). Some work has been recently done on several marine species (Fiala-Médioni & Métivier 1986, Fiala-Médioni et al. 1986, Beninger et al. 1988, Le Pennec et al. 1988), but very little is known about freshwater species ultrastructure apart from ciliary tract organisation (Way et al. 1989). Particularly the organ structure in *Corbicula fluminea* has been poorly investigated. To our knowledge, only a few studies on *Corbicula* histology do exist (Kraemer 1977, Kraemer & Lott 1977, Morton 1977 and 1982, Araujo et al. 1994) and more recently, an ultrastructural analysis on gill surface focused on cilia organisation (Way et al. 1989).

As we intend to use *Corbicula fluminea* as a model for freshwater experimental ecotoxicological studies, it was necessary to establish the normal structure and ultrastructure of the main organs involved in the uptake of environmental contaminants (heavy metals, pesticides, herbicides...) directly from the surrounding medium and/or through the food chain. Thus we selected gills and mantle with particular attention to the epithelia which necessarily control the ad- and absorption processes, and consequently the toxicological effects.

2. Materials and methods

2.1. Animals

Corbicula fluminea (average total weight = 1.019 ± 0.030 g; average shell length = $14,731 \pm 0.129$ mm) came from the «Canal du Midi», downstream from Toulouse (France). They were provided by the Laboratory of Hydrobiology from the University Paul Sabatier (Toulouse). In the laboratory, they were maintained in 40-litre tanks containing dechlorinated tap-water and a 6 cm-thick layer of substrate made of 50 % pure sand (SILAQ, 0.8 to 1.4 mm) and 50 % of sediment taken from the Garonne River banks, upstream from Bordeaux (homogenous silt rich in clays - 75-80 % - with a low TOC content, close to 2 %). They were fed 3 times a week with an unicellular algae suspension (*Scenedesmus* sp. and *Chlamydomonas* sp.), one dose corresponding to 3 µg chlorophyll-a per tank.

2.2. Chemicals

Glutaraldehyde and sodium cacodylate were from Merck. Osmium tetroxide came from Euromedex (France). Spurr resin was purchased from TAAB Ltd. All other chemicals were from the highest commercial grade available.

2.3. Sample preparation

For histological observations, *Corbicula fluminea* soft tissues (*i. e.* the entire body removed from the shell) were immersed *in toto* in the fixative (1 % glutaraldehyde in a sodium cacodylate 0.025 M buffer, pH 7.4) for 48 h at 4°C. They were rinsed in the sodium cacodylate 0.025 M buffer and dehydrated in graded ethanol, then treated with toluene and embedded in paraffin (58-60°C).

The sections (5 µm) were stained using nuclear fast red and picro-indigo-carmin topographic staining (Gabe 1968).

For electron microscopy study, *Corbicula fluminea* were rapidly dissected, immediately after being taken from the culture tank, and samples of gill and mantle were fixed in glutaraldehyde / sodium cacodylate fixative during 4 hours at 4°C. Several fixative solutions (from 1 % to 2.5 % glutaraldehyde, from 0.025 to 0.1 M sodium cacodylate) were tested. However, in accordance to the very low osmolality of *Corbicula fluminea*'s fluids (around 55 mOsm according to McCorkle & Dietz 1980), the best results were obtained with 1 % glutaraldehyde in a 0.025 M sodium cacodylate buffer (pH 7.4). They were rinsed in the sodium cacodylate 0.025 M buffer and post-fixed with 2 % osmium tetroxide in the same buffer.

They were then dehydrated in graded series of acetone and embedded in Spurr's resin (8 hours at 70°C). The ultrathin sections (70 nm) were contrasted by uranyl acetate / lead citrate (Reynolds 1963) and observed on a Jeol 100 S transmission electron microscope (60 kV) at the Electron Microscopy Center, University of Bordeaux I.

3. Results

3.1. General anatomy

A transverse histological middle section of the whole body shows the localization of the various tissues (Fig. 1). The soft body is covered by the mantle which lies directly under the shell. In the mantle cavity are located the gill ctenidia, each comprising an outer and a longer inner hemibranch, and the so-called visceral mass.

In the visceral mass, two main areas can be distinguished. Most of the visceral mass is occupied by the hermaphrodite gonad, the ovarian part representing the major part of it. In the center of the dorsal half of the visceral mass, the digestive gland is observed. The digestive tract comprises various sections including a large dorsal pouch-shaped stomach and a heavily folded intestinal tract showing numerous loops embedded in the digestive gland and the gonadal tissue.

3.2. Mantle

Different mantle areas are to be distinguished in relation to the distance from the margin. Near the edge of the shell, the mantle is very thick (Fig. 2), with muscles, and it gets thinner towards the median area (Fig. 3).

Most of the mantle is represented by a thin protective lamella (Fig. 3). It is composed of two epithelia, one facing the shell (outer epithelium), the other one facing the visceral mass (inner epithelium), separated by haemolymph sinuses and connective tissue. The outer epithelium is made of cubic epithelial cells (Fig. 4). The epithelial cells display a strong cytoplasmic segregation, and most of the cell is filled with glycogene particles. Most of the organelles including mitochondria and lysosomes are pulled towards the cell edges although some of them may be observed anywhere in the cytoplasm. The apical plasma membrane develops in microvilli. Loose connective tissue and large haemolymph lacunae (Fig. 5), containing circulating haemocytes and various cellular fragments (lysosomes, nuclei, membranes...), fill the interepithelial space. Some muscle fibres are sometimes observed. From place to place, the connective tissue gets more compact and forms septa supporting the interepithelial space (Figs 3

and 6). The inner epithelium (Fig. 7) is constituted of various cell types. The first are elongated club-shaped epithelial cells, with a basal nucleus. The cell content includes few mitochondria, small glycogen areas and lysosomes in the apical part of the cell and against the cell surface, small dense-bodies. The cell surface displays short microvilli. Another epithelial cell type is represented by smaller cubic cells, containing numerous mitochondria and displaying numerous long cilia. These cells are located in small crypts between the elongated epithelial cells. Some mucocytes are also observed.

The mantle margin structure is more complex. The mantle margin bears three folds separated by two grooves (Fig. 2). The outer fold epithelium is involved in the formation of the shell. The outer epithelium is made of prismatic cells with big plurilobate nuclei and short microvilli (Fig. 8). The cell content is very rich in more or less dense bodies and the upper part of the cells displays a great number of very small vesicles. In the space between the microvilli and the shell, some needle-shaped crystals (probably calcium carbonate) are observed. The periostracum is secreted by the epithelial cells of the inner face of the outer fold, inside the periostracal groove. In the deeper part of the groove (bulbous region: Fig. 9), the epithelial cells (basal cells) secrete the periostracal lamella which contributes to the building of the shell. The basal cell apical plasma membrane is infolded instead of displaying microvilli as the other epithelial cells do. The epithelial cells of the outer face of the outer fold (inner face of the periostracal groove) are elongated and display a great amount of heterogeneous vesicles. The medio-basal nucleus is elongated, with a central nucleolus. The cell surface presents more or less developed microridges and/or short microvilli. Towards the outside of the groove, the lamella gets thicker and the epithelial cell structure is different (Fig. 10). Two kinds of epithelia are observed, according to the side of the periostracal lamella they are facing. Immediately against the lamella which presents two layers, a dense layer and a loose fibrillous layer, on the side facing the calcareous part of the shell, the epithelium is made of very elongated cells, with a basal nucleus and small apical microvilli, resting on a very thick fibre layer. The cell content includes small vesicles, few mitochondria and dense tonofilament bunches. On the other side, the lamella is less dense and faces a thinner epithelium, made of small cubic cells, with a big central nucleus and small apical microridges. These cells present the same kind of vesicles as the basal cells from the bulbous region. The space between the epithelium and the lamella is filled with a heterogeneous clear fibrillous

Figs. 1-3.

Fig. 1. Transversal middle section in *Corbicula fluminea*. The soft body is covered with the mantle (M). The gill ctenidia are made of two hemibranches (Hb) on each side. The visceral mass is composed of the digestive gland (Dg) and the gonad (Go). The digestive tract is incorporated into the visceral mass and includes a dorsal gut (Gt) and numerous intestinal loops (I). Histological section. Fast nuclear red / picro-indigo-carmin. Bar scale = 300 μ m.

Fig. 1. Coupe transversale médiane de *Corbicula fluminea*. Le corps mou est recouvert du manteau (M). Les cténidies branchiales sont constituées de deux hémibranchies (Hb) de chaque côté. La masse viscérale est formée par la glande digestive (Dg) et la gonade (Go). Le tractus digestif est intriqué dans la masse viscérale et comprend un estomac dorsal (Gt) et de nombreuses boucles intestinales (I). Coupe histologique. Rouge nucléaire solide / picro-indigo-carmin. Echelle = 300 μ m.

Fig. 2. *Corbicula fluminea* mantle margin. The mantle margin has three folds (an outer Of, a middle Mf and an inner If fold) separated by two grooves, the outer groove (Og) secretes the periostracum (arrow). Hb = hemibranch; IE = Inner epithelium; Ig = inner groove; Mu = muscle; OE = outer epithelium. Histological section. Fast nuclear red / picro-indigo-carmin. Bar scale = 100 μ m.

Fig. 2. *Corbicula fluminea* : Zone marginale du manteau. Dans sa partie marginale, le manteau présente trois replis (un repli externe Of, un repli médian Mf et un repli interne If), séparés par deux sillons dont l'externe (Og) sécrète le périostacum (flèche). Hb = hémibranchie ; IE = épithélium interne ; Ig = sillon interne ; Mu = muscle ; OE = épithélium externe. Coupe histologique. Rouge nucléaire solide / picro-indigo-carmin. Echelle = 100 μ m.

Fig. 3. *Corbicula fluminea* median mantle area. The thin median area is composed of two thin epithelia, an outer (OE) and an inner (IE) epithelium separated by haemolymph sinuses (S) with circulating haemocytes (Hm) supported by collagen (Co). Histological section. Fast nuclear red / picro-indigo-carmin. Bar scale = 30 μ m.

Fig. 3. *Corbicula fluminea* : Zone médiane du manteau. La zone médiane est constituée de deux épithéliums très fins, un épithélium externe (OE) et un épithélium interne (IE) séparés par des lacunes (S) soutenues par du collagène (Co) et dans lesquelles circulent des hémocytes (Hm). Coupe histologique. Rouge nucléaire solide / picro-indigo-carmin. Echelle = 30 μ m.

matrix (Fig. 10). The second groove presents the same epithelium, with numerous vesicles and short microvilli, and a well developed cell coat (Fig. 11). The inner epithelium, facing the gill ctenidium is simpler than the epithelium from the mantle median area (Figs. 7 and 12). The epithelial cells are of the same type as the elongated cells from the inner epithelium described above. Some ciliated cells are observed, but no crypts are formed yet. The space comprised between the epithelia is occupied by muscle fibres embedded in a collagen matrix (Fig. 13).

3.3. Gills

The general anatomy of *Corbicula fluminea* gills is that of Eulamellibranch Bivalves, which has been already described (Beninger et al. 1988, Le Pennec et al. 1988). It consists of two rows of folded filaments on each side of the body constituting the ctenidium. Inside the fold, the filaments delimitate an interlamellar chamber communicating with the external medium through ostia (Fig. 14).

From a structural point of view, the gill epithelium is divided into two main areas, a ciliated frontal area, and an unciliated abfrontal area (Fig. 14). In each area, for each cell type, the distance between haemolymph and water (appreciated as the cell width) was measured.

The abfrontal area represents the respiratory part of the filament. It is located on the inner side of the hemi-

branch fold (Fig. 14), in the interlamellar chamber, and it is constituted of a unique layer of thin pavement respiratory epithelial cells (Fig. 15). The largest part of the cell ($3.5 \pm 0.3 \mu$ m) is the area where the nucleus is located. On each side, the cell develops thin cytoplasmic extensions ($0.4 \pm 0.1 \mu$ m). The cell surface displays small microridges and a well developed cell coat (Fig. 15). The cell content includes mitochondria and rough endoplasmic reticulum, few lysosomes and vesicles. In the same area, some mucocytes are observed, particularly in the interfilament junctions, but they remain quite rare (Fig. 16).

The ciliated area presents various cell types corresponding to 3 sub-areas, from back to front: the lateral, latero-frontal and frontal areas. The following description is based on the observation of transversal sections of hemibranches, although longitudinal and sagittal sections were also observed for a better knowledge of cilia organisation.

The lateral area is constituted of two cell types, very well differentiated, and rather different from a structural point of view. The first one ($4.9 \pm 0.4 \mu$ m) directly in contact with the respiratory epithelium is represented by a single epithelial cell totally covered with cilia, the lateral cilia (Fig. 17). The electron-dense cell content includes very numerous round mitochondria, glycogen, an elongated nucleus and some lysosomes, both located in the very basal area. The cilia do not

Figs. 4-7.

Fig. 4. *Corbicula fluminea* median mantle area. The outer epithelium (facing the shell) is made of cubic epithelial cells, with cytoplasmic segregation. The organelles including mitochondria (mi) are generally pulled to the cell edges while the cell is filled with glycogen particles (Gly). The apical plasma membrane develops microvilli (mv). T.E.M. Uranyl acetate/lead citrate. Bar scale = 2 μ m.

Fig. 4. *Corbicula fluminea* : Zone médiane du manteau. L'épithélium externe, qui fait face à la coquille, est constitué de cellules épithéliales cubiques présentant une ségrégation cytoplasmique. Les organites, et particulièrement les mitochondries (mi), sont repoussés vers la périphérie de la cellule tandis que le cytoplasme se caractérise par sa grande richesse en particules de glycogène (Gly). La membrane plasmique apicale développe des microvillosités (mv). M.E.T. Acétate d'uranyle / Citrate de plomb. Echelle = 2 μ m.

Fig. 5. *Corbicula fluminea* median mantle area. Haemolymph sinuses are present between the epithelia among loose connective tissue (Cn). Circulating haemocytes (Hm) and various cellular fragments including lysosomes, nuclei and membranes, are visible. T.E.M. Uranyl acetate/lead citrate. Bar scale = 10 μ m.

Fig. 5. *Corbicula fluminea* : Zone médiane du manteau. Entre les deux épithéliums se trouvent des lacunes ménagées au sein d'un tissu conjonctif lâche (Cn). Des hémocytes (Hm) et différents fragments cellulaires tels que des lysosomes, des noyaux et des fragments de membrane circulent dans ces lacunes. M.E.T. Acétate d'uranyle / citrate de plomb. Echelle = 10 μ m.

Fig. 6. *Corbicula fluminea* median mantle area. The haemolymph sinuses are supported by collagen (Co) septa. T.E.M. Uranyl acetate/lead citrate. Bar scale = 5 μ m.

Fig. 6. *Corbicula fluminea* : Zone médiane du manteau. Les lacunes sont soutenues par des septums de collagène (Co). M.E.T. Acétate d'uranyle / citrate de plomb. Echelle = 5 μ m.

Fig. 7. *Corbicula fluminea* median mantle area. The inner epithelium (facing the visceral mass) is made of different cell types including ordinary epithelial cells (Ec) bearing short microvilli (mv), ciliated epithelial cells (Cc) developing cilia bunches (C) in small crypts (*) and mucocytes (Mc). The epithelium lies on a thick collagen layer (Co). Gly = glycogen. T.E.M. Uranyl acetate/lead citrate. Bar scale = 5 μ m.

Fig. 7. *Corbicula fluminea* : Zone médiane du manteau. L'épithélium interne, qui fait face à la masse viscérale, est constitué de différents types cellulaires : des cellules épithéliales ordinaires (Ec) qui portent de courtes microvillosités (mv), des cellules épithéliales ciliées (Cc) qui développent des faisceaux de cils (C) dans des petites cryptes (*) et des mucocytes (Mc). L'épithélium repose sur une épaisse couche de collagène (Co). Gly = glycogène. M.E.T. Acétate d'uranyle / citrate de plomb. Echelle = 5 μ m.

present a particular organisation: whatever the direction of the section, they are always covering the cell surface. A single but often dichotomous long microvillum is present between two cilia.

The neighbour cell is the only not ciliated cell in this part of the gill epithelium and shows a much lower electronic density than the ciliated cells (Fig. 18). Compared to the other gill epithelial cells, it displays a well developed rough endoplasmic reticulum and is very rich in vesicles and in tubular structures. This cell is described as a «secretory cell». It is 4.36 ± 0.2 μ m wide and its surface develops in small microridges, covered with a well developed cell coat.

Beside the secretory cell are the latero-frontal epithelial cells. Their main characteristic is the presence of two parallel rows of cilia (latero-frontal cilia), following a dorso-ventral axis (Fig. 19). The cell surface also develops in long, sometimes dichotomous, microvilli, displaying a well developed cell coat. The cell content includes lysosomes and mitochondria, as well as glycogene and a few small pinocytotic vesicles in the very apical part of the cell, the big nucleus being located at the basis of the cell. These cells (8.4 ± 0.5 μ m) and their neighbour cells, the frontal epithelial cells (11.3 ± 1.5 μ m), constitute the thickest area of the gill epithelium.

Contrary to the latero-frontal cilia, the frontal cilia present no actual organisation. Each cell displays several cilia randomly scattered on its surface (Fig. 20). The frontal cells are more narrow and more elongated than the latero-frontal cells. However, their content is similar, except for the presence of small dense bodies in the very apical part of the frontal cells (Fig. 20). The cell nucleus is oval-shaped, elongated, and located in the basal part of the cell.

The gill structure is supported by a fibre rod underlining the epithelium. This fibre layer is very thick in the ciliated area but gets very thin and finally almost disappears in the respiratory area (Figs 15 and 20).

4. Discussion

So far, the internal anatomy of *Corbicula fluminea* has been poorly investigated. Earlier microscopic studies were based upon histological approaches (Kraemer 1977, Kraemer & Lott 1977) and gave no information about the cytological structure of the various epithelia.

4.1 Mantle

To our knowledge, few information exists on bivalve mantle ultrastructure except some studies on the involvement of mantle margin in shell formation (Neff

Figs. 8-10.

Fig. 8. *Corbicula fluminea* mantle margin. The outer epithelium (facing the shell) is made of prismatic epithelial cells developing short microvilli (mv) at the apical surface. The big multi-lobed nucleus (N) occupies a great part of the cell. Small needle-shaped crystals (arrow) can be observed at the cell surface, where the microvilli are in contact with the shell. T.E.M. Uranyl acetate/lead citrate. Bar scale = 2 μ m.

Fig. 8. *Corbicula fluminea* : Zone marginale du manteau. L'épithélium externe, qui fait face à la coquille, est constitué de cellules épithéliales prismatiques qui développent de courtes microvillosités (mv) à leur surface. Le noyau (N), volumineux et plurilobé, occupe une grande partie de la cellule. Des petits cristaux en forme d'aiguille (flèche) peuvent être observés à la surface des cellules, là où les microvillosités sont au contact de la coquille. M.E.T. Acétate d'uranyle / citrate de plomb. Echelle = 2 μ m.

Fig. 9. *Corbicula fluminea* mantle margin. The outer (periostracal) groove is involved in the periostracum formation. In the deepest part of the groove (bulbous region) the epithelial cells secrete the periostracal lamella (P). The cell content is very rich in small heterogeneous vesicles (Ve). The apex of the cell is covered with short microvilli (mv). T.E.M. Uranyl acetate/lead citrate. Bar scale = 5 μ m.

Fig. 9. *Corbicula fluminea* : Zone marginale du manteau. Le sillon externe (sillon périostracal) est impliqué dans la sécrétion du périostracum. Dans la partie la plus profonde du sillon (région bulbeuse), les cellules épithéliales sécrètent la lamelle périostracale (P). Le cytoplasme est très riche en petites vésicules au contenu hétérogène (Ve). L'apex de la cellule est couvert de courtes microvillosités (mv). M.E.T. Acétate d'uranyle / citrate de plomb. Echelle = 5 μ m.

Fig. 10. *Corbicula fluminea* mantle margin. In the outer part of the periostracal groove, the periostracal lamella (P) has thickened and presents as two distinct areas: a very dense lamella covered with a fibrillous layer. The epithelia on each side of the lamella are different. On the electron-dense side, the epithelium is made of columnar cells with elongated nuclei, containing dense tonofilament bunches (arrow). On the other side, the epithelium is constituted of cubic epithelial cells with big nuclei (N) occupying most of the cell. Both epithelia display apical microvilli (mv). V = haemolymph vessel. T.E.M. Uranyl acetate/lead citrate. Bar scale = 10 μ m.

Fig. 10. *Corbicula fluminea* : Zone marginale du manteau. Dans la partie la plus externe du sillon périostracal, la lamelle périostracale (P) s'épaissit et présente deux zones distinctes : une lamelle très dense couverte d'une couche fibrillaire. Les épithéliums de part et d'autre de la lamelle sont différents. Du côté de la zone dense aux électrons, l'épithélium est constitué de cellules colonnaires avec des noyaux allongés, contenant des faisceaux denses de tonofilaments (flèche). De l'autre côté, l'épithélium est formé de cellules épithéliales cubiques dont le noyau volumineux (N) occupe la majeure partie. Les deux épithéliums présentent des microvillosités apicales (mv). V = vaisseau hémolympatique. M.E.T. Acétate d'uranyle / citrate de plomb. Echelle = 10 μ m.

1972, Bubel 1976, Petit et al. 1979 and 1980, Araujo et al. 1994, Reindl & Hazprunar 1996).

The structures observed in *Corbicula fluminea* mantle margin are similar to those described in freshwater (Bubel 1976, Petit et al. 1979 and 1980) as well as marine species (Neff 1972). The periostracum originates from the inner face of the outer fold. This mechanism has been elucidated in various species including *Mercenaria mercenaria* (Neff 1972), *Anodonta cygnea* (Bubel 1972), and *Amblema amblema perplicata* (Petit et al. 1979). The outer face is involved in calcium carbonate transport, as demonstrated by the presence of crystalline needles above the epithelial cell microvilli, in the periostracal *cul-de-sac* fluid. Petit et al. (1980) showed that these elementary crystalline needles present between the epithelium and the shell were trapped within the mesh of a fibrous organic network, leading to the formation of spherical subunits which will associate into mature prisms (shell prismatic layer). However, the main purpose of our study was not to investigate the role of the mantle margin in the shell formation in *Corbicula fluminea*, but to describe the normal structure of the epithelia likely to be involved in contaminant uptake and/or damaged by those contaminants.

Apart from its involvement in shell formation, the main role of the mantle is to cover and protect the body. However, the very thin inner epithelium of the medium area constitutes a particularly narrow barrier between the water and the haemolymph. Moreover, the cilia bunches scattered all over the inner epithelium surface create water movement involved in the exchanges. In accordance to its structure the mantle is thought to be involved in gas and ionic exchanges as well as nutrition in various bivalves including *Corbicula fluminea* (Henry & Mangum 1980, Britton & Morton 1982, Deaton 1982, Henry & Saintsing 1983). It could then also play a great part in contaminant uptake and its functions as an exchange barrier could be heavily perturbed by toxic xenobiotics.

4.2. Gill

Gill structure has been more investigated than mantle structure (Peck 1977, Morton 1977 and 1989), particularly on marine bivalves (Moore 1971, Ribelin & Collier 1977, Fiala-Medioni & Métivier 1986, Fiala-Medioni et al. 1986, Beninger et al. 1988, Le Pennec et al. 1988, Beninger et al. 1993, 1994 and 1995). However, yet little is known about freshwater species (Nakao 1975, Kraemer 1977 and 1983; Way et al. 1989, Pandey & Datta Munshi 1991).

Figs. 11-13.

Fig. 11. *Corbicula fluminea* mantle margin. The epithelial cells of the inner groove are similar to the periostracal groove epithelial cells, containing numerous vesicles (Ve) and displaying short microvilli (mv). The epithelium rests on a thick collagen layer (Co). Mu = muscle fibers. T.E.M. Uranyl acetate/lead citrate. Bar scale = 10 μ m.

Fig. 11. *Corbicula fluminea* : Zone marginale du manteau. Les cellules épithéliales du sillon interne sont semblables à celles du sillon périostacal, contenant de nombreuses vésicules (Ve) et présentant de courtes microvillosités apicales (mv). L'épithélium repose sur une épaisse couche de collagène (Co). Mu = fibres musculaires. M.E.T. Acétate d'uranyle / citrate de plomb. Echelle = 10 μ m.

Fig. 12. *Corbicula fluminea* mantle margin. The inner epithelium, facing the body, is nearly similar to the inner epithelium of the thin median area. The epithelial cells (Ec) are of the same type as the elongated epithelial cells described earlier (see figure 7). Note the thick collagen layer (Co) under the epithelium. mv = microvilli; N = nucleus. T.E.M. Uranyl acetate/lead citrate. Bar scale = 5 μ m.

Fig. 12. *Corbicula fluminea* : Zone marginale du manteau. L'épithélium interne, qui fait face à la masse viscérale, est à peu près similaire à celui de la zone médiane. Les cellules épithéliales (Ec) sont du même type que les cellules allongées décrites plus haut (voir Fig. 7). On remarque l'épaisse couche de collagène (Co) sous l'épithélium. mv = microvillosités ; N = noyau. M.E.T. Acétate d'uranyle / citrate de plomb. Echelle = 5 μ m.

Fig. 13. *Corbicula fluminea* mantle margin. The muscle fibre bunches (Mu) are separated with a collagen matrix (Co). T.E.M. Uranyl acetate/lead citrate. Bar scale = 10 μ m.

Fig. 13. *Corbicula fluminea* : Zone marginale du manteau. Les faisceaux de fibres musculaires (Mu) sont séparées par une matrice de collagène (Co). M.E.T. Acétate d'uranyle / citrate de plomb. Echelle = 10 μ m.

Corbicula fluminea gill is known as an Eulamelli-branch gill, meaning that the gill filaments are joined by interlamellar and interfilamentous junctions so that the lamellae actually consists of solid sheets of tissue (Barnes 1987). In most of the species studied by previous investigators (Moore 1971, Ribelin & Collier 1977, Beninger et al. 1988, Le Pennec et al. 1988, Beninger et al. 1993, 1994, 1995), the gill is a filibranch gill where the interfilamentous junctions do not exist. Moreover, most of those species have plicate gills composed of at least two types of filaments, the ordinary filaments and the principal filament lying between the folds (Beninger et al. 1988, Le Pennec et al. 1988). In *Corbicula fluminea* only ordinary filaments were observed. Moreover, the respiratory epithelium is very much simpler in *Corbicula fluminea* than reported for plicate gills where ciliated cells were observed in the abfrontal area (Moore 1971, Le Pennec et al. 1988). The epithelial cells are little differentiated and they build a very thin wall between water and haemolymph. This supposes that the main function of this area consists in simple passive exchanges between water and haemolymph, with no implication either in water movement or in particle capture.

The ciliated lateral and frontal areas are highly differentiated, indicating a great specialization of each kind of cell type. All the ciliated cells (lateral, latero-frontal and frontal cells) are involved in water and particle movements (Le Pennec et al. 1988, Way et al. 1989). The need for energy for cilia movements of these cells is indicated by their richness in mitochondria, particu-

larly in the lateral ciliated cells which surface is covered with cilia and content extremely rich in mitochondria. According to the cilia organisation, *Corbicula fluminea* can be placed in the Macrociliobranchia group as defined by Atkins (1938) and Owen (1978). In this group, the latero-frontal tracts are composed of compound eu-latero-frontal cirri together with one or more rows of subsidiary pro-latero-frontal cilia. Each cirrus arises from a single cell and consists of cilia arranged in two parallel rows, as observed in *Corbicula fluminea*. In the oyster *Crassostrea gigas*, Ribelin and Collier (1977) distinguished a latero-frontal cirrus and a para-latero-frontal cirrus, the latter made of very closed spaced, double row of cilia originating from a single row of cells lying on either side of the frontal ciliary tract. However, only one latero-frontal cirrus was present on either side of the filament in *Corbicula fluminea*.

Another kind of very differentiated cells is present in the lateral area. These cells are unciliated and their content presents a highly developed tubular system and numerous mitochondria. They were previously described as secretory cells (Britton & Morton 1982), involved in mucus secretion for particle transport. However, proper mucocytes have been identified in the respiratory area and no mucous cell was observed in the ciliated area in *Corbicula fluminea*, although goblet cells has been found in the frontal area in other species like the oyster *Crassostrea gigas* (Ribelin & Collier 1977). The mucocyte structure is completely different from the secretory cell structure. Moreover, the secretory

Figs. 14-16.

Fig. 14. *Corbicula fluminea* gill. A middle transversal section in an outer hemibranch reveals the filaments delimiting an interlamellar chamber (ILC) communicating with the external medium through ostia (O). The epithelium is divided into two main areas : a respiratory area (Re) in the interlamellar chamber and a ciliated area (Ce) outwards, displaying three groups of cilia : the lateral (Lc), latero-frontal (Lfc) and frontal (Fc) cilia. The inner hemibranch epithelium displays the same structure. Hv = haemolymph vessel; ILC = interlamellar chamber. Histological section. Nuclear fast red / picro-indigo-carmin. Bar scale = 30 μ m.

Fig. 14. *Corbicula fluminea* : Branchie. La coupe transversale médiane d'une hémibranchie externe montre les filaments qui délimitent une chambre interlamellaire (ILC) communiquant avec le milieu externe par des ostiums (O). L'épithélium est divisé en deux zones principales : une zone respiratoire (Re) dans la chambre interlamellaire et une zone ciliée (Ce) vers l'extérieur, montrant trois groupes de cils : les cils latéraux (Lc), latéro-frontaux (Lfc) et frontaux (Fc). L'épithélium de l'hémibranchie interne présente la même structure. Hv = vaisseau hémolympatique ; ILC = chambre interlamellaire. Coupe histologique. Rouge nucléaire solide / picro-indigo-carmin. Echelle = 30 μ m.

Fig. 15. *Corbicula fluminea* gill. The respiratory epithelium is made of thin pavement epithelial cells displaying small microridges (mr). The supporting rod (Sr) tends to disappear in this area. Hv = haemolymph vessel; mi = mitochondria; N = nucleus. T.E.M. Uranyl acetate/lead citrate. Bar scale = 2 μ m.

Fig. 15. *Corbicula fluminea* : Branchie. L'épithélium respiratoire est formé de cellules épithéliales pavimenteuses très aplaties, présentant des microrides (mr). La structure de soutien (Sr) tend à disparaître dans cette zone. Hv = vaisseau hémolympatique ; mi = mitochondrie ; N = noyau. M.E.T. Acétate d'uranyle / citrate de plomb. Echelle = 2 μ m.

Fig. 16. *Corbicula fluminea* gill. Some small mucocytes are present between the epithelial cells of the respiratory epithelium. md = mucus droplet. T.E.M. Uranyl acetate/lead citrate. Bar scale = 2 μ m.

Fig. 16. *Corbicula fluminea* : Branchie. Quelques petits mucocytes peuvent être observés entre les cellules épithéliales de l'épithélium respiratoire. md = gouttelette de mucus. M.E.T. Acétate d'uranyle / citrate de plomb. Echelle = 2 μ m.

cells display the same structure as the chloride cells described in fish gills (Sardet et al, 1979, Wendelaar-Bonga et al. 1990). Therefore they could be cells involved in active exchanges between water and haemolymph, particularly ionic exchanges. However, so far, no description of this kind of "chloride cells" was found in the literature, neither in marine, nor in freshwater species. Actually, although most of marine species are known to be isosmotic to their environment over the entire range of their salinity tolerance (Deaton 1982), *Corbicula fluminea* has been found to be able to osmoregulate at salinities below 13 ‰ for periods up to 7 days (Morton & Tong 1985). Particularly the presence of Na^+/K^+ -ATPase activities involved in osmoregulation was demonstrated in gills, mantle and kidney of several freshwater bivalve species including *Corbicula fluminea* (Deaton 1982). However, so far, further studies should be necessary to relate this ability to regulate to the presence of these ionocyte-like cells.

5. Conclusion

This study focused on the epithelia of *Corbicula fluminea* organs including gill and mantle. These biological barriers, characterized by a very large exchange area and specific structures, are involved in fundamental and physiological functions (respiration, osmoregu-

lation, nutrition). From an ecotoxicological point of view, they represent the primary sites of interaction with the bioavailable contaminants from the surrounding medium (water column, sediment porewater) and/or the food ingested. Thus, they control the ad- and absorption mechanisms and jointly the toxicological effects induced at their level as well as in the other organs and tissues, via the exchanges between the haemolymph and the internal compartments. The knowledge of the structure and ultrastructure of these biological barriers is therefore of high importance for the interpretation of bioaccumulation and toxicity mechanisms. It will be useful for the analysis of the results from experimental studies set up in our laboratory in order to investigate mercury and cadmium transfers from the water column and sediment as initial contamination sources, under the combined effects of several abiotic factors (temperature, pH, dissolved oxygen, salinity...).

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Figs. 17-20.

- Fig. 17. *Corbicula fluminea* gill. The lateral ciliated epithelial cell is covered with cilia (Lc) separated by long microvilli (mv). The cell content is very rich in mitochondria (mi) and displays small but numerous glycogen areas (Gly). T.E.M. Uranyl acetate/lead citrate. Bar scale = 2 μ m.
- Fig. 17. *Corbicula fluminea* : Branchie. La cellule épithéliale ciliée latérale est couverte de cils (Lc) séparés par de longues microvillosités (mv). Le contenu cellulaire est très riche en mitochondries (mi) et montre des plages de glycogène (Gly) de petit taille mais très nombreuses. M.E.T. Acétate d'uranyle / citrate de plomb. Echelle = 2 μ m.
- Fig. 18. *Corbicula fluminea* gill. The «secretory» cell (chloride cell ?) is not ciliated and its cytoplasm presents a very low electron density partially due to its richness in small vesicles (Ve). Er = endoplasmic reticulum; Lc = lateral cilia; mi = mitochondria; mr = microridges. T.E.M. Uranyl acetate/lead citrate. Bar scale = 1 μ m.
- Fig. 18. *Corbicula fluminea* : Branchie. La cellule sécrétrice (cellule à chlorures ?) n'est pas ciliée et son cytoplasme présente une faible densité aux électrons en partie due à sa richesse en petites vésicules (Ve). Er = réticulum endoplasmique ; Lc = cils latéraux ; mi = mitochondrie ; mr = microrides. M.E.T. Acétate d'uranyle / citrate de plomb. Echelle = 1 μ m.
- Fig. 19. *Corbicula fluminea* gill. A low magnification electron micrograph of a filament transverse section allows recognition of the various types of epithelial cells : lateral ciliated cell (L), latero-frontal (Lf) and frontal (F) epithelial cells, displaying their respective cilia (Lc, Lfc, and Fc). The lateral ciliated cell is totally covered with the lateral cilia (Lc), although the latero-frontal cilia (Lfc) are arranged in two parallel rows and the frontal cilia (Fc) present no particular arrangement. Note the position of the secretory cell (Sc) located between the lateral ciliated cell and the latero-frontal cells. In this area, the supporting rod (Sr) gets very thick. T.E.M. Uranyl acetate/lead citrate. Bar scale = 5 μ m.
- Fig. 19. *Corbicula fluminea* : Branchie. A faible grandissement, on peut distinguer sur une coupe transversale du filament branchial les différents types cellulaires présents : cellules ciliées latérale (L), latéro-frontales (Lf) et frontales (F), montrant leurs groupes de cils respectifs (Lc, Lfc, et Fc). La cellule latérale ciliée est complètement recouverte par les cils latéraux (Lc), tandis que les cils latéro-frontaux (Lfc) sont alignés sur deux rangées parallèles et que les cils frontaux (Fc) ne présentent aucune organisation particulière. On remarque la position de la cellule sécrétrice (Sc) localisée entre la cellule latérale ciliée et les cellules latéro-frontales. Dans cette zone, la structure de soutien (Sr) devient très épaisse. M.E.T. Acétate d'uranyle / citrate de plomb. Echelle = 5 μ m.
- Fig. 20. *Corbicula fluminea* gill. The apex of the frontal epithelial cells (F) displays small dense bodies (D) underneath the plasma membranè. F = frontal epithelial cell; Fc = frontal cilia; mv = microvilli; N = nucleus. T.E.M. Uranyl acetate/lead citrate. Bar scale = 10 μ m.
- Fig. 20. *Corbicula fluminea* : Branchie. L'apex des cellules épithéliales frontales (F) montre des inclusions denses de petite taille (D) sous la membrane plasmique. F = cellule épithéliale frontale ; Fc = cils frontaux ; mv = microvillosités ; N = noyau. M.E.T. Acétate d'uranyle / citrate de plomb. Echelle = 10 μ m.

References

- Abbe G.R. & Sanders J.G. 1990. — Pathways of silver uptake and accumulation by the American oyster (*Crassostrea virginica*) in Chesapeake Bay. *Est. Coast. Shelf Sci.*, 31 : 113-123.
- Abbe G.R., Sanders J.G. & Riedel G.F. 1994. — Silver uptake by the oyster (*Crassostrea virginica*) : effect of organism size and storage sites. *Est. Coast. Shelf Sci.*, 39 : 249-260.
- Araujo R., Moreno D. & Ramos, M.A. 1993. — The asiatic clam *Corbicula fluminea* (Müller, 1774) in Europe. *Amer. Malacol. Bull.*, 10 (1): 39-49.
- Araujo R., Ramos M.A. & Bedoya J. 1994. — Microtubules in the shell of the invasive bivalve *Corbicula fluminea* (Müller, 1774) (Bivalvia: Heterodonta). *J. Mollusc. Studies*, 60 (4): 405-413.
- Atkins D. 1938. — On the ciliary mechanisms and interrelationships of lamellibranches. *Quat. J. microsc. Sci.*, 79 : 181-308.
- Barnes R.D. 1987. — Invertebrate zoology. Saunders College Publishing. Fifth Edition.
- Beninger P.G., Dwiono S.A.P. & Le Pennec M. 1994. — Early development of the gill and implications for feeding in *Pecten maximus* (Bivalvia Pectinidae). *Mar. Biol.*, 119 : 405-412.
- Beninger P.G., Le Pennec M. & Salaün M. 1988. — New observations of the gills of *Placopecten magellanicus* (Mollusca: Bivalvia) and implications for nutrition. 1. General anatomy and surface microanatomy. *Mar. Biol.*, 98 (1) : 61-70.
- Beninger P.G., Potter T.M. & St-Jean S.D. 1995. — Paddle cilia fixation artefacts in pallial organs of adult *Mytilus edulis* and *Placopecten magellanicus* (Mollusca, Bivalvia). *Can. J. Zool.*, 73 (3): 610-614.
- Beninger P.G., St-Jean S., Poussart Y. & Ward J.E. 1993. — Gill function and mucocytes distribution in *Placopecten magellanicus* and *Mytilus edulis* (Mollusca: Bivalvia): the role of mucus in particle transport. *Mar. Ecol. Prog. Ser.*, 98 : 275-282.
- Britton J.C. & Morton B. 1982. — A dissecting guide, field and laboratory manual for the introduced bivalve *Corbicula fluminea*. *Malacol. Rev.*, Suppl. 3 : 82 pp.
- Bubel A. 1976. — An electron microscope study of the formation of the periostracum in the freshwater bivalve *Anodonta cygnea*. *J. of Zool.*, 180 : 211-232.
- Campbell J. & Evans R.D. 1991. — Cadmium concentrations in the freshwater mussel (*Elliptio complanata*) and their relationship to water chemistry. *Arch. of Environ. Contam. Toxicol.*, 20 : 125-131.
- Couillard Y., Campbell P.G.C. & Tessier A. 1993. — Responses of metallothionein concentrations in a freshwater bivalve (*Anodonta grandis*) along an environmental cadmium gradient. *Limnol. Oceanogr.*, 38 (2): 299-313.
- Deaton L.E. 1982. — Tissue (Na+K)-activated adenosine triphosphatase activities in freshwater and brackishwater bivalve molluscs. *Mar. Biol. Lett.*, 3 : 107-112.

- Doherty F.G., Failla M.L. & Cherry D.S. 1988. — Metallothionein-like heavy metal binding protein levels in the Asiatic clams are dependent on the duration and mode of exposure to cadmium. *Wat. Res.*, 22 (7) : 927-932.
- Fiala-Médioni A. & Métivier C. 1986. — Ultrastructure of the gill of the hydrothermal vent bivalve *Calymene magnifica* with a discussion of its nutrition. *Mar. Biol.*, 90 : 215-222.
- Fiala-Médioni A., Métivier C., Herry A. & Le Pennec M. 1986. — Ultrastructure of the gill of the hydrothermal vent mytilid *Bathymodiolus* sp. *Mar. Biol.*, 92 : 65-72.
- Gabe M. 1968. — *Techniques histologiques*. Masson éd. Paris : 1113 pp.
- Henry M., Boucaud-Camou E. & Lefort Y. 1991. — Functional microanatomy of the digestive gland of the scallop *Pecten maximus* (L.). *Aquat. Liv. Resour.*, 4 (3) : 191-202.
- Henry R.P. & Mangum C.P. 1980. — Salt and water balance in the oligohaline clam *Rangia cuneata*. 1. Anisotonic extracellular regulation. *J. exp. Zool.*, 211 (1) : 1-10.
- Henry R.P. & Saintsing D.G. 1983. — Carbonic anhydrase activity and ion regulation in three species of osmoregulating bivalve molluscs. *Physiol. Zool.*, 56 (2) : 274-280.
- Ishii T., Okoshi K., Otake T. & Nakhara M. 1992. — Concentrations of elements in tissues of four species of Tridacnidae. *Nippon Suisan Gakkaishi*, 58 (7) : 1285-1290.
- Kraemer L.R. 1977. — Aspects of the functional morphology of the mantle/shell and mantle/gill complex of *Corbicula* (Bivalvia: Sphaeriacea). *Bull. Amer. Malacol. Union*, 1977 : 25-31.
- Kraemer L.R. & Lott S. 1977. — Microscopic anatomy of the visceral mass of *Corbicula* (Bivalvia: Sphaeriacea). *Bull. Amer. Malacol. Union*, 1977 : 48-56.
- Le Pennec M., Beninger G.P. & Herry A. 1988. — New observations of the gills of *Placopecten magellanicus* (Mollusca Bivalvia) and implications for nutrition. II. Internal anatomy and microanatomy. *Mar. Biol.*, 98 : 229-237.
- McCloskey J.T., Newman M.C. & Dixon P.M. 1995. — Effect of metal and metalloid contaminated sediment on the spatial distribution of Asiatic clams (*Corbicula fluminea*). *Arch. Environ. Contam. Toxicol.*, 28 : 203-208.
- McCorkle S & Dietz T.H. 1980. — Sodium transport in the freshwater Asiatic clam *Corbicula fluminea*. *Biol. Bull. Mar. Biol. Lab. Woods Hole*, 159 (2) : 325-336.
- McMahon R.F. 1982. — The occurrence and spread of the introduced Asiatic freshwater clam *Corbicula fluminea* (Müller) in North America: 1924-1982. *The Nautilus*, 96 : 134-141.
- Mersch J. & Johansson L. 1993. — Transplanted aquatic mosses and freshwater mussels to investigate the trace metal contamination in the rivers Meurthe and Plaine, France. *Environ. Technol.*, 14 : 1027-1036.
- Mersch J., Morhain E. & Mouvet C. 1993. — Laboratory accumulation and depuration of copper and cadmium in the freshwater mussel *Dreissena polymorpha* and the aquatic moss *Rhynchostegium riparioides*. *Chemosphere*, 27 (8) : 1475-1485.
- Moore H.J. 1971. — The structure of the laterofrontal cirri on the gill of certain lamellibranch molluscs and their role in suspension feeding. *Mar. Biol.*, 11 : 23-27.
- Morton B. 1977. — The influence of inflammatory granulomas in the ctenidial marsupium of *Corbicula fluminea* (Mollusca: Bivalvia): a consequence of larval incubation. *J. Invert. Path.*, 30 : 5-14.
- Morton B. 1982. — Some aspects of the population structure and sexual strategy of *Corbicula* cf. *fluminalis* (Bivalvia: Corbiculoidea) from the Pearl River, People's Republic of China. *J. Mollusc. Stud.*, 48 : 1-23.
- Morton B. 1989. — The functional morphology of the organs of the mantle cavity of *Batissa violacea* (Lamarck, 1797) (Bivalvia: Corbiculacea). *Am. Malac. Bull.*, 7 (1) : 73-79.
- Morton B. & Tong K.Y. 1985. — The salinity tolerance of *Corbicula fluminea* (Bivalvia: Corbiculoidea) from Hong-Kong. *Malacol. rev.*, 18 : 91-95.
- Mouthon J. 1981. — Sur la présence en France et au Portugal de *Corbicula* (Bivalvia: Corbiculidae) originaires d'Asie. *Basteria* 45 : 109-116.
- Nakao T. 1975. — The fine structure and innervation of gill lamellae in *Anodonta*. *Cell Tiss. Res.*, 157 : 239-254.
- Neff J.M. 1972. — Ultrastructural studies of periostracum formation in the hard shelled clam *Mercenaria mercenaria* (L.). *Tiss. Cell*, 4 : 311-326.
- Owen G. 1978. — Classification and the bivalve gill. *Phil. Trans. R. Soc. London Ser. B*, 284 : 377-385.
- Palmork K.H. & Solbakken J.E. 1981. — Distribution and elimination of [9-14C]phenanthrene in the horse mussel (*Modiola modiolus*). *Bull. Environ. Contam. Toxicol.*, 26 (2) : 196-201.
- Pandey A. & Datta Munshi J.S. 1991. — Respiratory metabolism and scanning electron microscopy of the gills of freshwater riverine mussel *Parreysia favidens*. *J. Freshwater Biol.*, 3 (1) : 65-70.
- Peck R.H. 1877. — The minute structure of the gills of Lamellibranch Mollusca. *Quart. Journ. of Microsc. Sci.*, 17 : 43-66.
- Petit H., Davis W.L. & Jones R. 1979. — Morphological studies on the periostracum of the fresh-water mussel *Amblema* (Unionidae): light microscopy, transmission electron microscopy and scanning electron microscopy. *Tiss. Cell*, 11 : 633-642.
- Petit H., Davis W.L., Jones R.G. & Hagler H.K. 1980. — Morphological studies on the calcification process in the freshwater mussel *Amblema*. *Tiss. Cell*, 12 (1) : 13-18.
- Reindl S. & Hazprunar G. 1996. — Fine structure of caeca and mantle of Arcoid and Limopsoid Bivalves (Mollusca: Pteriomorpha). *The Veliger*, 39 (2) : 101-116.
- Reynolds E.S. 1963. — The use of lead citrate at high pH as electron-opaque stain in electron microscopy. *J. Cell Biol.*, 17 : 208-212.
- Ribelin B.W. & Collier A. 1977. — Studies on gill ciliation of the American oyster *Crassostrea virginica* (Gmelin). *J. Morphol.*, 151 : 439-450.
- Roesijadi G. & Unger M.E. 1993. — Cadmium uptake in gills of the mollusc *Crassostrea virginica* and inhibition by calcium channel blockers. *Aquat. Toxicol.*, 24 : 195-206.
- Sardet C., Pisam M. & Maetz J. 1979. — The surface epithelium of teleostean fish gills. Cellular and junctional adaptations of the chloride cell in relation to salt adaptation. *J. Cell Biol.*, 80 : 96-117.
- Skul'sky I.A., Burovina I.V., Vasilyeva V.F., Lukyanova O.N., Nikiforov V.A. & Syasina I.G. 1989. — Uptake and microlocalization of cadmium in marine bivalve mollusc tissues. *Comp. Biochem. Physiol.*, 92C (2) : 349-353.
- Tessier A., Couillard Y., Campbell P.G.C. & Auclair J.C. — 1993. Modeling Cd partitioning in oxic lake sediments and Cd concentrations in the freshwater bivalve *Anodonta grandis*. *Limnol. Oceanogr.*, 38 (1) : 1-17.
- Viarengo A. & Nott J.A. 1993. — Mechanisms of heavy metal cation homeostasis in marine invertebrates. *Comp. Biochem. Physiol.*, 104C : 355-372.
- Way C.M., Hornbach D.J., Deneka T. & Whitehead R.A. 1989. — A description of the ultrastructure of the gills of freshwater bivalves, including a new structure, the frontal cirrus. *Can. J. Zool.*, 67 : 357-362.
- Wendelaar-Bonga S.E., Flik G., Balm P.H.M. & van der Meij J.C.A. 1990. — The ultrastructure of chloride cells in the gills of the teleost *Oreochromis mossambicus* during exposure to acidified water. *Cell Tiss. Res.*, 259 : 575-585.
- Zarogian G., Yevitch P. & Anderson S. 1993. — Effect of selected inhibitors on cadmium, nickel and benzo(a)pyrene uptake in brown cells of *Mercenaria mercenaria*. *Mar. Environ. Res.*, 35 : 41-45.